GUIDELINES FOR THE CONTROL OF TUBERCULOSIS IN ELEPHANTS

Compiled by the
NATIONAL TUBERCULOSIS WORKING GROUP FOR ZOO & WILDLIFE SPECIES
(November 1997)

Introduction

In response to recent occurrences of disease due to Mycobacterium tuberculosis in captive elephants, the following preliminary guidelines for the testing, treatment, and surveillance of M.tb complex (M. tuberculosis, M.bovis, M. africanum, M. microti) in elephants have been created. These guidelines should be used with the understanding that neither the ancillary diagnostic tests nor treatment regimens have been completely validated in elephants. As more information becomes available, it is anticipated that these guidelines will change. Ancillary tests may be incorporated in future diagnostic requirements if reliable correlations to actual infection with pathogenic strains of Mycobacteria can be documented. At this time, it is recommended that the diagnostic guidelines not be given the status and force of state regulations except in the case of requiring culture samples for M.tb complex organisms as outlined.

General Testing

In order to adequately address the concerns of TB in the general elephant population, all captive elephants should be tested by culture. Since early information indicates that ancillary diagnostic tests (skin test, BTB, ELISA, etc.) have questionable correlation to true disease status, only the results of cultures and exposure to known culture positive animals should be used to determin e treatment regimens and travel restrictions until other tests are validated. Cultures will facilitate identification of elephants that are actually shedding live organisms and represent a potential threat to both animal and human health.

There is little information currently available about the reliability of antemortem tests for the diagnosis of tuberculosis in elephants. To collect data necessary to determine the sensitivity and specificity of diagnostic tests, it is strongly recommended that all elephants, particularly those that are culture positive or have been exposed to a culture positive animal, be tested using ancillary diagnostic test such as the BTB, CSU ELISA, ISU ELISA, comparative skin test, and nucleic acid amplification tests. Results will be used to develop improved strategies for diagnosis and management of tuberculosis in elephants in the future.

Bacteriologic Culturing

Samples for culturing should be collected under the direct visual supervision of a licensed veterinarian. These should consist of three samples from the trunk taken on separate mornings within a one week period. Samples should be taken after water has been witheld for at least 2 hours to reduce sample dilution and contamination. Several methods may be used to collect the samples depending on acceptance of the procedure by the elephant. Of the following three methods, the trunk wash with bag seems to provide the most effective way to gather samples at this time.

1. Trunk wash with bag (or other suitable container) - Using a catheter tip syringe, instill 60 ml sterile saline into the trunk. Immediately place a clean, one gallon plastic bag over the end of the trunk and hold in place until the animal exhales into the bag. Transfer 20 ml of the sample to a sterile leakproof container.

2. Trunk wash - Using a 14 french feeding tube, introduce 30-60 ml of sterile saline into the trunk then aspirate. Transfer 20 ml of the sample into leakproof container.

3. Trunk swab - Insert culturette into trunk and swab. Obtain a minimum of 2-3 swabs per elephant for each sample.

Samples should be immediately frozen or refrigerated until ready for shipment. (If the three samples are to be held and shipped at one time, samples should be frozen immediately.) Samples should be placed in leakproof containers and double-bagged. For shipment, samples should be placed on ice packs and shipped immediately by overnight express to NVSL or other laboratory facility offering comparable procedures for identification of Mycobacteria. When submitting samples to NVSL, use the APHIS 10-4 (8004) submission form. Request mycobacterial culture with speciation. (Positive cultures from laboratories that do not have the capability to differentiate M.tb complex organisms should be forwarded to NVSL for speciation.) Acid-fast stains and nucleic acid amplification tests are also highly recommended.

TB Management Groups (A,B,C,D)

Based upon the culture results (for M.tb complex) and status of exposure to known culture positive animals, all elephants should fall into one of the following four management groups: (Note: These groups are intended for initial classification only. Follow-up procedures for future years are outlined in each group.)

Group A (Negative culture; No exposure to culture positive animal in last 5 years)

Monitor annually by three culture sample method on separate mornings over a one week period. (Ancillary tests such as BTB, ELISA, and skin testing are encouraged for data collection but not required.) No treatment or travel restrictions.

Group B (Negative culture; Exposure to culture positive animal between 1-5 years ago)

Monitor quarterly by the three culture method for one year and then annually thereafter if all cultures remain negative. No treatment or travel restrictions. (Ancillary tests such as BTB, ELISA, and skin testing are encouraged for data collection but not required.)

Group C (Negative culture; Exposure to culture positive animal within last 12 months)

This group should be tested and then handled according to either Option 1 (Treatment) or Option 2 (No treatment) as follows:

Testing

1. As soon as initial culture results are known, reculture by 3 sample method over a one week period.
2. Perform ancillary diagnostic tests: (strongly recommended for data gathering but not required)
   A. Acid fast stain on trunk sample smear.
   B. BTB test
   C. CSU ELISA test
   D. ISU ELISA test
   E. Nucleic acid amplification test
   F. Comparative skin test with balanced PPD (avian/bovis)

Note: Collect all blood samples before injecting tuberculin for skin test. Tuberculin may interfere with some test results.

Option 1 - Treatment

After testing is completed (samples taken and skin test read) treatment should begin as follows:

1. Isoniazid (INH) daily for two months then 4x/wk for 4 months at a dosage sufficient to achieve a minimal blood level of 0.5 ug/ml. **
2. Rifampin daily for two months then 4x/wk for 4 months at a dosage sufficient to achieve a minimal blood level of 0.5 ug/ml. **
3. Vitamin B6 (pyridoxine) at 1mg/kg daily for 6 months to counter possible peripheral neuropathy due to INH.

** For most current dosages, contact Dr. Susan Mikota. Dosages may differ depending on route of administration.

Travel restrictions

Option 1 elephants should not travel for the first two months of treatment. At the end of two months of treatment and confirmation of negative reculture result (sampled just prior to treatment), these animals may travel if they receive the next four months of treatment on the road.

Monitoring

1. During the 6 months of treatment, monthly blood tests (CBC/blood chemistries) should be conducted to monitor general health and liver enzymes. (INH may cause liver damage. In addition, leukopenia has occured in at least one elephant apparently due to INH toxicity.)
2. Beginning with the onset of treatment, samples should be collected by the three culture sample method quarterly for eighteen months. If all cultures are negative, collect three culture samples annually thereafter.

Option 2 -No treatment

If treatment is not elected, Group C elephants should be restricted from traveling for 12 months. During this time, samples for culture should be collected by the three culture sample method every other month for one year. If all cultures are negative, collect three culture samples annually thereafter.

Group D (Current culture positive)

These elephants should be separated from other elephants and the public until follow-up cultures are negative. This group should be tested and treated as follows:

Testing

Perform ancillary diagnostic tests: (recommended)

1. Acid-fast stain
2. BTB test
3. CSU ELISA test
4. ISU ELISA test
5. Nucleic acid amplification
6. Comparative skin test with balanced PPD (avian/bovis)

Note: Collect all blood samples before injecting tuberculin for skin test. Tuberculin may interfere with some test results.

Treatment

1. Isoniazid (INH) daily for 2 months then 4x/wk for 10 months at a dosage sufficient to achieve a blood level of 0.5 ug/ml. **
2. Rifampin daily for 2 months then 4x/wk for 10 months at a dosage sufficient to achieve a blood level of 0.5 ug/ml.**
3. Pyrazinamide (PZA) daily for 2 months. **
4. Vitamin B6 (pyridoxine) at 1 mg/kg daily for 12 months to counter possible peripheral neuropathy due to INH.

** For most current dosages, contact Dr. Susan Mikota. Dosages may differ depending on route of administration.

Travel restrictions

No travel permitted until 6 months of treatment has been completed and two consecutive negative cultures have been obtained. Before release for travel, the attending veterinarian must certify that the elephant appears clinically normal and that adequate blood levels of treatment drugs have been obtained.

Monitoring

1. During the 12 months of treatment, monthly blood tests (CBC/blood chemistries) should be conducted to monitor general health and liver enzymes. (INH may cause liver damage. Leukopenia has occured in at least one elephant apparently due to INH toxicity.)
2. During treatment, three culture samples should be taken each month until there are two consecutive months that have negative results. After two consecutive months show all negative cultures, culture sampling may be reduced to every other month.
3. After treatment is completed, reculture every other month for one year using the three sample method, then annually thereafter if all cultures remain negative.

ALL GROUPS (A,B,C,D): If an elephant is too difficult to handle, a restraint crush is not available, and testing cannot be done safely without the aid of chemical restraint, that elephant should only be tested opportunistically when restrained for other purposes. If an elephant cannot be tested safely, it should not be permitted to have direct public contact or be moved from the home facility.

NECROPSY

ALL ELEPHANTS THAT DIE SHOULD BE NECROPSIED AND EXAMINED FOR TUBERCULOSIS REGARDLESS OF EXPOSURE STATUS!!! Send representative sections of any gross lesions and a complete set of tissues (lung, liver, spleen, mesenteric lymph nodes, bronchial lymph nodes, and other major organs) fixed in formalin to NVSL in Ames, IA and the National Zoo. An additional complete set of tissues for culture should be sent frozen to NVSL. See attached complete necropsy protocol for further details.

EMPLOYEE SAFETY AND HEALTH

ALL employees that are in contact with elephants should be tested for TB annually following established human testing guidelines. All new employees should be tested prior to contact with elephants. Any employee with a currently positive sputum culture should not be in contact with elephants.

Any facility housing a known positive culture (M.tb complex) animal should develop a program to protect employees from TB exposure, including the use of face masks and proper disinfection procedures..

INSTRUCTIONS FOR TEST PROCEDURES

1. CULTURE SUBMISSION - Send trunk cultures to NVSL either frozen or on icepacks by overnight express. Containers should be leakproof and double-bagged. Use the APHIS Form 10-4 for submission. Cost: $25/sample for processing plus minimum of $50 for speciation. To establish an account for billing contact Connie Osmundson (515)239-8571.

2. BTB TEST - Notify lab (Texas A&M) 48 hrs. before sampling to schedule tests. Handle samples very carefully. Try not to stress animals during collection. Use one 15 ml or two 10 ml green top heparinized tubes; fill tubes completely and rock gently 15-20 times to prevent clotting. Also, take one 5 ml sample in a red top serum separator tube. Label tubes with waterproof marker. Pack in cushioned box with cold packs, making sure cold packs do not touch tubes. Send by overnight delivery. Cost: about $100/sample.

3. ELISA TEST - Need 4 ml of frozen serum for each test; send overnight on ice packs to ISU and CSU. Cost: Varies. CSU currently is not charging. ISU test runs from ~$30-60 depending on the number of antigens used.

4. NUCLEIC ACID AMPLIFICATION - Collect trunk samples in 2ml sterile tubes; ship overnight on dry ice to NVSL or other suitable laboratory.

5. COMPARATIVE SKIN TEST - Must use balanced PPD (avian/bovis). Veterinarians must receive specialized training before administering this test. (At this time, no training courses are scheduled but they may become available in the future thru USDA, Veterinary Services.)

DISCUSSION

Ancillary test results (BTB, ELISA, etc.) will be compared with culture and necropsy results over the coming years to determine their validity. Please forward all test results to AAZV for compilation. Banking of frozen serum prior to tuberculin testing and treatment is recommended to facilitate future research needs when new tests are introduced.

TESTING LABORATORIES

1. Cultures - National Veterinary Services Laboratories (NVSL)
Dr. Janet Payeur / Jerry Jarnagin
1800 Dayton Rd.
Ames, IA 50011
(515) 239-8676 or 239-8548

2. Histopathology -1. Dr. Richard Montali
Department of Pathology
National Zoo
3001 Connecticut Ave.
Washington, DC 20008
(202) 673-4869 Fax: (202) 673-4660

2. NVSL
Dr. Art Davis
Chief or Pathobiology
1800 Dayton Rd.
Ames, IA 50011
(515) 239-8521

3. BTB - Texas A&M University
Dept. of Veterinary Pathology
Room 215 VMS Bldg.
College Station, TX 77843
(409) 845-4640 Fax: (409) 862-1088

4. ELISA (ISU) - Dr. Charles Thoen
Iowa State University
Department of Microbiology, Immunology & Preventive Medicine
College of Veterinary Medicine Complex
16th Street SW
Ames, IA 50011
(515) 294-7608 Fax: (515) 294-8500

5. ELISA (CSU) -Delphi Chatterjee
Colorado State University
Dept. of Microbiology
Ft. Collins, CO 80523-1677
(970) 491-7495 Fax: (970) 491-1815

6. Nucleic Acid Amplification Test - Send to NVSL (see above address) or other suitable laboratory. Call ahead to ensure test availability.

CONTACTS FOR QUESTIONS

1. Dr. Susan Mikota - Audubon Center for Research of Endangered Species (ACRES)
14001 River Rd.
New Orleans, LA 70131
(504) 398-3111 Fax: (504) 398-3100 E-mail: smikota@acres.org

2. Dr. Miava Binkley - USDA, APHIS, Animal Care
Suite 302
2568-A Riva Rd.
Annapolis, MD 21401
(410) 571-8692 Fax: (410) 224-2854 E-mail: mbinkley@aphis.usda.gov

3. Dr. Joel Maslow - Boston VA Medical Center
150 S. Huntington Ave.
Boston, MA 02130
(617) 232-9500 x 5576 Fax: (617) 232-2969
E-mail: maslow.joel n dr@boston.va.gov

CONTACT FOR NECROPSY/PATHOLOGY QUESTIONS

1. Dr. Dick Montali - National Zoo
Department of Pathology
3001 Connecticut Ave., NW
Washington, DC 20008
(202) 673-4869 Fax: (202) 673-4660 E-mail: nzp em 026@sivm.si.edu

CONTACT FOR COLLECTING TEST RESULTS

American Association of Zoo Veterinarians
See attached reporting form.

Tuberculosis Surveillance Report Form

Institution: _____
Submmiter: _____
Address: _____
_____
Tel: _____ Fax: _____ email: _____

Species: _____ ISIS # _____ Other ID: _____

Age: ___ [ ]actual [ ]estimate Sex: [ ]male [ ]female

EXPOSURE STATUS:
[ ]This is a known infected animal
[ ]There is a known exposure to culture positive source within the past 12 months
[ ]There is a known exposure to a culture positive within the past 1-5 years
[ ]There is no known exposure to a culture positive source in the last 5 years

CULTURE RESULTS

Source	Method	Test Date	Lab	Result

Source: trachea; trunk; oropharynx; other
Method: swab; tracheal wash; trunk wash; trunk wash w/ bag; other
Results: negative; M.tb complex; M.tb; M. bovis; M. avium; other (list)
Note: Results reported as M.tb complex do not differentiate between M.tb and M.bovis.

ACID FAST STAIN

Source	Test Date	Lab	Result

BTB RESULTS
Date of last skin test:

Test Date	Lab	BTB Result	ELISA Result	LT Result

Terms for results: Bovine; Avian; Equivocal; No data

ELISA RESULTS

Date of last skin test:

Lab	Test Date	Result

AMPLIFICATION TESTS

Test	Sample source	Test Date	Lab	Result
PCR				
MTD				

IDENTIFICATION TESTS

Test	Sample source	Test Date	Lab	Result
RFLP				

INTRADERMAL TEST (Comparative test preferred)

Type of tuberculin	**Amount**	**Site**	**Test Date**	**Result at 72 hours**

Site: Pinnae; ear fold;caudal fold; cervical
Result: Negative, suspect;

BIOPSY:Clean and disinfect the injection site after the tuberculin test is read. Remove a plug of skin with attached dermis from the center of the site (a 5 mm biopsy punch works well). Fix in 10 % buffered formalin and submit to Dr. Montali; National Zoo, 3001 Connecticut Ave. Washington DC.20008-2598 Include skin test and biopsy dates.

Send completed form to:
Dr. Wilbur Amand
American Association of Zoo Veterinarians
6 North Pennell Rd
Media PA 19063
T:610-892-4812; F:610-891-4813' email: 75634.235@compuserve.com

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
NATIONAL VETERINARY SERVICES LABORATORIES
AMES, IOWA 50010

SPECIMEN SUBMISSION

INSTRUCTIONS: Use a separate request for each species and each owner/broker. See reverse of Part 5 for definitions *(item 11)* and instructions for identification *(item 19)*.

PAGE ___ OF ___

1. NAME OF SUBMITTER *(Last, First, MI)*

MAILING ADDRESS *(Street, City, and State, Zip code)*

Telephone Number ( )

2. NAME OF OWNER/BROKER *(Last, First, MI)*

CITY

STATE

3. LOCATION OF ANIMALS

County

State

4. HERD/FLOCK SIZE

5. NO. IN HERD/FLOCK AFFECTED

6. NO. IN HERD/FLOCK DEAD

7. EXAMINATIONS REQUESTED *(Diseases and Test Procedures)*

8. COLLECTED BY

9. DATE COLLECTED

10. AUTHORIZED BY

11. PURPOSE OF SUBMISSION *("X" one) (See reverse side of Part 5 for definitions)*

☐ General Diagnostic ☐ Surveillance/Monitor ☐ Import
☐ FAD/EP Diagnostic ☐ Developmental/Research ☐ Export
☐ NVSL Intralab Diagnostic ☐ Reagent Evaluation

12. COUNTRY OF ORIGIN/DESTINATION

13. REFERRAL NUMBER

14. PRESERVATION *("X" applicable item(s))*

☐ None ☐ Ice Pack ☐ Dry Ice ☐ Formalin ☐ Borax ☐ Alcohol ☐ Other *(specify)*

15. SPECIMENS SUBMITTED *("X" applicable item(s))*

☐ Blood ☐ Feces ☐ Parasite ☐ Serum ☐ Tissue ☐ Whole Bird
☐ Culture ☐ Feed ☐ Plant ☐ Soil ☐ Urine ☐ Other *(specify)*
☐ Extract ☐ Milk ☐ Semen ☐ Swab ☐ Water

16. TOTAL NUMBER OF SPECIMENS SUBMITTE

17. SPECIES OR SOURCE *("X" one)*

☐ Cattle ☐ Goat ☐ Environment ☐ Chicken ☐ Other (specify)
☐ Swine ☐ Horse ☐ Reagent ☐ Turkey
☐ Sheep ☐ Donkey ☐ Pet Bird (specify)

18. NUMBER OF SHIPPING CONTAINERS USED

19. NUMBER OF ANIMALS SAMPLED

20. BREED

21. IDENTIFICATION *(see reverse side of Part 5)*		AGE	SEX	IDENTIFICATION *(see reverse side of Part 5)*		AGE	SEX
Sample ID	Animal ID/Breed			Sample ID	Animal ID		

22. ADDITIONAL DATA *(History, clinical signs, post mortem findings, remarks, tentative diagnosis, etc. Use additional sheets if necessary. Attach 5 copies.)*

23. SIGNATURE OF SUBMITTER AND DATE SUBMITTED

NVSL USE ONLY

CONDITION	PRIORITY	DISTRIBUTION	RECEIVED BY

NVSL ACCESSION NO.

BTb Submission Form

(This form may be reproduced)

Samples must be booked 24hrs prior to blood collection (phone: 409-845-4640)
This form must be filled out completely to facilitate reporting of results.
Please print legibly.

Date blood was collected_____

Owner or Contact Individual	Veterinarian
Farm Name	Hospital/Organization
Mailing Address	Mailing Address
City/State Zip	City/State Zip
Home Phone	Phone
Work Phone	
Fax	Fax
Owner or Contact Individual Signature	Veterinarian Signature

Please indicate billing address: Owner:_____ Veterinarian_____

Please indicate preferred method of reporting: Mail:_____ Fax:_____

Please indicate if owner is a member of NADeFA: Yes:_____ No:_____

Tag No	Species	Retest (Y/N)	Age	Sex (M/F)	Last ST Date	Last ST Result	Stressed (Y/N)	Sedated (Y/N)

Tag No	Species	Retest (Y/N)	Age	Sex (M/F)	Last ST Date	Last ST Result	Stressed (Y/N)	Sedated (Y/N)

ADDENDUM

Please add the following laboratory for ELISA testing to the TB guidelines:

Dr. Mo Salman
Department of Environmental Health
Colorado State University
Environmental Health Bldg., Rm. 107
Ft. Collins, CO 80523-1676
Attn: Joni

Phone: (970) 491-2379
Fax: (970) 491-2940

Please use the attached submission form to send samples. Shipping boxes must say "Refrigerate upon arrival".

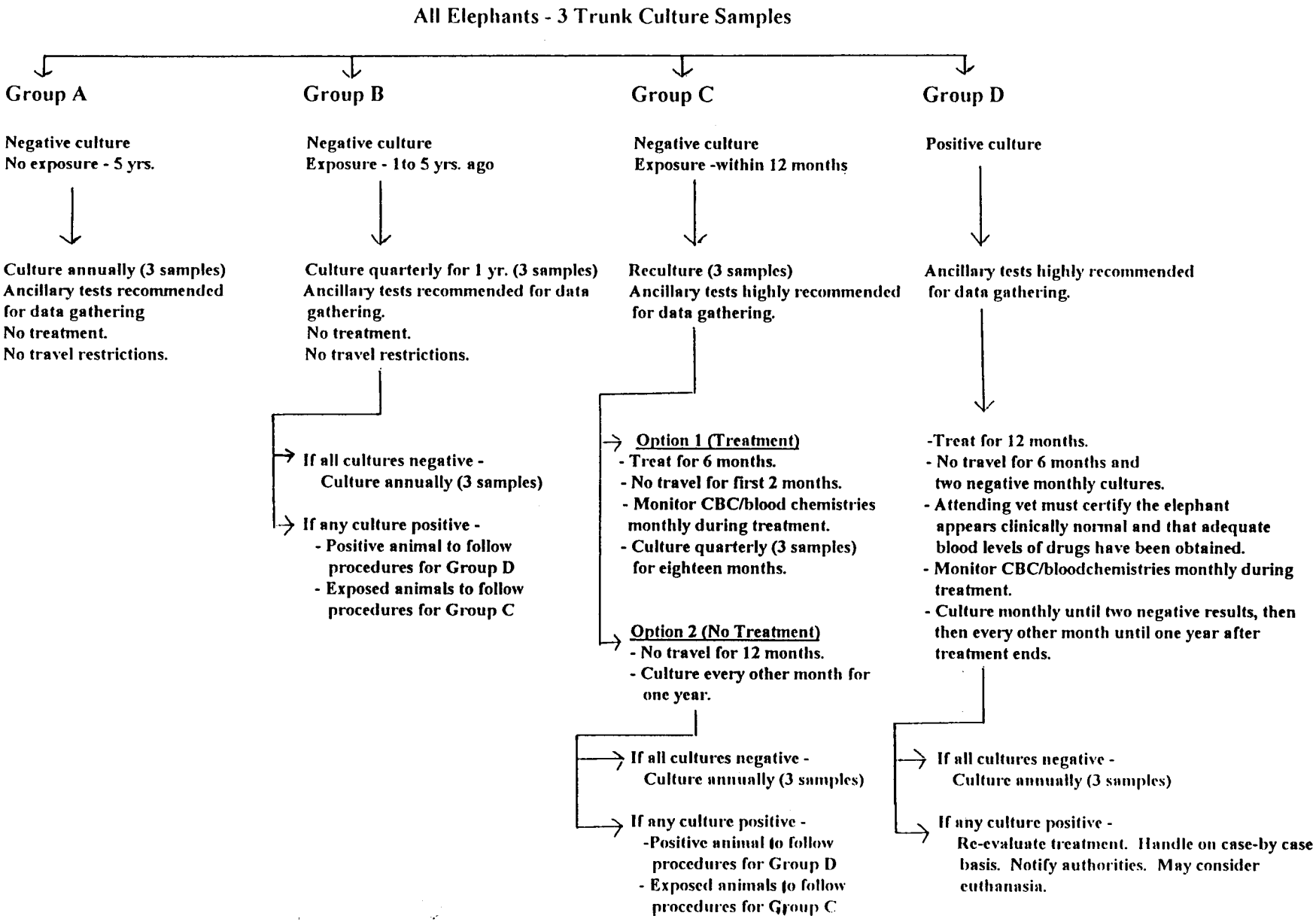
TB ELISA SAMPLE SUBMISSION FORM					PAGE ____ of ____	
Mycobacterium bovis Testing Laboratory Dr. Mo Salman Department of Environmental Health Colorado State University Environmental Health Bldg, RM 107 Ft. Collins, CO 80523-1676 Attn: Joni Phone: 970/491-2379 Fax: 970/491-2940					LAB USE ONLY: JTPID: _____ DATE RC'D: _____ LOCATION: _____	
VETERINARIAN:					HERD OWNER:	
CLINIC:					NUMBER OF SAMPLES SUBMITTED:	
ADDRESS:					PHONE:	
CITY:			STATE:		ZIP CODE:	
ANIMAL ID	SEX	AGE	SPECIES	TB SKIN TEST RESULTS	OTHER TB TEST RESULTS	DATE SAMPLE TAKEN

SAMPLE SUBMISSION

1. Samples must be collected by a licensed veterinarian.

2. Shipping boxes must say "Refrigerate Upon Arrival."

3. Collect blood into red top clotting tube, allow to clot, centrifuge, transfer serum into another vial, and send at 4 C or frozen.


All Elephants - 3 Trunk Culture Samples
Group A
Negative culture
No exposure - 5 yrs.
Culture annually (3 samples)
Ancillary tests recommended for data gathering
No treatment.
No travel restrictions.
Group B
Negative culture
Exposure - 1to 5 yrs. ago
Culture quarterly for 1 yr. (3 samples)
Ancillary tests recommended for data gathering.
No treatment.
No travel restrictions.
If all cultures negative - Culture annually (3 samples)
If any culture positive -
- Positive animal to follow procedures for Group D
- Exposed animals to follow procedures for Group C
Group C
Negative culture
Exposure -within 12 months
Reculture (3 samples)
Ancillary tests highly recommended for data gathering.
Option 1 (Treatment)
- Treat for 6 months.
- No travel for first 2 months.
- Monitor CBC/blood chemistries monthly during treatment.
- Culture quarterly (3 samples) for eighteen months.
Option 2 (No Treatment)
- No travel for 12 months.
- Culture every other month for one year.
If all cultures negative - Culture annually (3 samples)
If any culture positive -
-Positive animal to follow procedures for Group D
- Exposed animals to follow procedures for Group C
Group D
Positive culture
Ancillary tests highly recommended for data gathering.
-Treat for 12 months.
- No travel for 6 months and two negative monthly cultures.
- Attending vet must certify the elephant appears clinically normal and that adequate blood levels of drugs have been obtained.
- Monitor CBC/bloodchemistries monthly during treatment.
- Culture monthly until two negative results, then then every other month until one year after treatment ends.
If all cultures negative - Culture annually (3 samples)
If any culture positive -
Re-evaluate treatment. Handle on case-by case basis. Notify authorities. May consider euthanasia.